IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Richard Ian Christopherson et al.
Application No.	:	09/888,959
Filed	:	June 25, 2001
For	:	AN ASSAY TO DETECT A BINDING PARTNER

Examiner	:	Anne L Holleran
Art Unit	:	1642
Docket No.	:	650061.401C1
Date	:	September 13, 2004

DECLARATION UNDER 37 C.F.R. § 1.132
OF RICHARD IAN CHRISTOPHERSON

Commissioner for Patents
Washington, D.C. 20231

I, Richard Ian Christopherson, hereby declare:

1. I am currently Professor in the School of Molecular and Microbial Biosciences at the University of Sydney, Maze Crescent, Sydney, New South Wales, 2006, Australia. My Curriculum Vitae attached hereto as Exhibit A, which includes a listing of publications that I have authored or co-authored and which evidence my expertise *inter alia* in flow cytometric technology and cancer diagnosis.

2. I am a co-inventor of U.S. Patent Application No. 09/888,959 (hereinafter referred to as "USSN '959"). The claimed subject matter relates to an assay for identifying a type of leukemia by contacting a biological sample from a patient with an array of immunoglobulin molecules bound to a solid support. The assay steps require the

interaction of antigens present on the surface of the cell in the biological sample with those immunoglobulin molecules bound to the solid support. A diagnosis is then performed by establishing a pattern of interaction that is a differential pattern of density providing an identifiable signal. The differential pattern identified is determinative of either the absence of leukemia or the presence of a specific type of leukemia.

3. The ability to distinguish between a multitude of different leukemias requires the examination of multiple surface markers. For example, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HCL), B cell lymphoma (BCL), and acute myeloid leukemia (AML) may be distinguished using a panel of antibodies specific for different cell surface antigens (*see* USSN '959, page 64, lines 9-14; Table 7). This analysis is possible due to the nature of the claimed method, which allows for the concurrent detection and comparison of at least seven cell surface antigens, as many as about fifty cell surface antigens (*see, e.g.*, page 44, lines 24-30; Tables 1-7); and as many as about 1000 antigens (*see, e.g.*, page 35, lines 9-18) concurrently within a single assay.

4. I have reviewed the Office Action dated June 3, 2004, and the documents cited therein in the rejection under 35 U.S.C. § 103 has been explained to me by patent advisors. The Action alleges that the presently claimed method would have been obvious to a person having ordinary skill in the art over Chang (U.S. Patent No. 4,591,570) in light of Valet et al. (*Cytometry* 30:275-288, 1997); Terstappen et al. (U.S. Patent No. 5,234,816); Verwer et al. (U.S. Patent No. 5,605,805); or Orfao de Matos Correira E Val (U.S. Patent No. 5,538,855). The Action also alleges that the claimed method would have been obvious to a person having ordinary skill in the art over Hoeffler (U.S. Publication No. 2002/0164656) in view of Terstappen (U.S. Patent No. 5,234,816).

5. I submit, as a highly skilled artisan, that a person having ordinary skill in the art would not have been motivated to combine the teachings of Chang with any of the cited documents according to the teachings therein to achieve the claimed method with an expectation of success. Chang (U.S. Patent No. 4,591,570) can be contextualized by

reference to Chang (*J. Immunol. Meth.* 65 (1983) 217-223). These references merely describe a method using three antibodies immobilized on a solid support for the purpose of determining the proportion of specific subsets of B-cells, T-cells and monocytes in the mononuclear cell fraction. One type of antibody was used to measure each of these subsets, there was no use or mention of patterns of recognition, protein expression profiling or disease signatures. Moreover, as a skilled artisan, I submit that I would not have found that Chang motivated me in any way to use an array of Chang's design to identify or monitor *any* type of disease state. Chang also does not mention or suggest that binding of an array of immunoglobulins to different cell surface antigens results in any pattern whatsoever such as a pattern of interaction that is a differential pattern of density that provides an identifiable signal. The differential pattern of interaction indicates the relative density of interaction between each immunoglobulin and its cognate cell surface antigen, which may result, for example, from differential density of cells that bind to a discrete spot on the array, the differential expression of particular antigens, and/or the number of antigens per cell (*see, e.g.,* specification, page 26, lines 11-20). The identifiable signal therefore may vary not only with a particular cell type such as a different type of leukemia but may vary with the level of expression of a particular antigen on different cell types, which results in a pattern of interaction that may be visualized and/or quantified *(see, e.g.,* specification, Tables 1-8). Chang is completely silent on this critical aspect that provides a unique identifiable signal, likely because it was as unknown to him as others of skill in the art at the time of filing the present application.

Further, we do not consider that the methods of Chang can be reproduced or practiced by one of skill in the relevant art. Specifically, we have replicated the experiments described in working examples of Chang and have found that the data obtained are not reliable. We have found, like others, that antibodies adhered directly to glass do not reliably remain adhered to the surface of the glass unless the surface is first derivatised. The derivatisation of glass surfaces to anchor ligands is technology which was not available at the time of filing of USSN '959 and has only subsequently been

developed. Accordingly, when using the assay device described in Chang to determine the presence of specific antigens on a cell surface, one would not be able to differentiate between a cell population which is negative for the surface antigen(s) being tested versus a negative result due to the loss of antibodies from the surface of the glass slide during cell capture and subsequent washing. Similarly, it is difficult to block acid-washed glass with a protein solution such as skim milk.

6. To demonstrate the unsatisfactory outcome of the experiments disclosed in Chang (1983, 1986) the following experiments were performed. The experiment was run on 29 June 1999 by Ms Odetta de la Vega and Dr Larissa Belov. Briefly, the method of Chang (1983, 1986) was tested in the following experiment. The slides used were Oncyte slides with a nitrocellulose film from Molecular Probes (Eugene OR, manufactured by Grace Biolabs, Bend OR), silanated (amine) slides from Telechem International (Sunnyvale, CA) and plain glass microscope slides (acid washed). Antibody dots (200 µL, 200 µg/mL) were applied to the 3 types of glass slides and dried for 60 minutes. The slides were immersed in phosphate-buffered saline (PBS) and then in skim milk (5% w/v Diploma, Melbourne, Australia) for 15 min at 37°C. The slides were dried for 60 min at room temperature, and then stored dry at 4°C.

Mononuclear leukocytes were prepared from 50 ml of normal blood by centrifugation (1,600 rpm, 600 g, 30 min, room temperature) on 30 mL of Histopaque. Mononuclear leukocytes were collected at the interface, washed twice in Hanks solution and resuspended in Hanks at a density of 10^7 cells/mL. The slides with antibody dots were moistened in PBS and then 500 µL of the leukocyte suspension (normal peripheral blood leukocytes) was placed on the array on the slide and incubated at 37°C for 30 min. The slides were gently washed in PBS to remove unbound cells and observed microscopically. The Oncyte slides gave a well defined dot pattern for normal peripheral blood leukocytes (mononuclear fraction) which was photographed using 25-fold magnification with Nomaski optics. There was no result from the Telechem or plain glass slides due to high background binding of leukocytes to the glass between the

antibody dots. The nitrocellulose film of the Oncyte slides could be blocked preventing non-specific cell binding while satisfactory blocking was not obtained with the silanated or plain glass slides.

7. A type of leukemia cell may be identified using the claimed method by determining the differential pattern of interaction between discrete regions of immobilized immunoglobulins and cells in a biological sample, which is indicated by the relative interaction between each immunoglobulin and its cognate cell surface antigen. This pattern of interaction may result, for example, from differential density of cells that bind to a discrete spot on the array, the differential expression of particular antigens, and/or the number of antigens per cell (*see, e.g.,* specification, page 26, lines 11-20). The resulting pattern of interaction may be visualized as an image and/or quantified in another manner that indicates a differential signal, such as the +/- system, or the 8-bit greyness scale (1-256) used by the Medsaic Array Reader, illustrated in the present application *(see, e.g.,* specification, Tables 1-8).

8. Each of Valet et al., Terstappen et al., Verwer et al., and Orfao de Matos Correira E Val teaches flow cytometric methods for distinguishing different types of leukemias or lymphoid populations by detection of CD antigens expressed on a cell. By way of background, flow cytometry is the analysis of biological material by detection of the light-absorbing or fluorescing properties of cells passing in a narrow stream through a laser beam. These properties can depend on the auto-fluoresecence of a cell population, or alternatively, fluorochromes can be bound to the cells that produce signals at different wavelengths. An optical absorbance or fluorescence profile of the sample is then produced. At the date of filing of the present application, typical flow cytometers could detect three fluorochromes concurrently on cells in a single sample.

9. I submit that a person having ordinary skill in the art at the time of filing USSN '959 would not have been motivated by any suggestion or teaching in Chang, or with any teaching regarding flow cytometry in Valet et al., Terstappen et al., Verwer et

al., and Orfao de Matos Correira E Val to achieve the claimed method. Chang teaches a general method for analyzing several antibody-antigen binding interactions but fails to provide any suggestion or motivation for using the method taught therein to construct an extensive array of immobilized antibodies for identifying a type of leukemia or any disease in a human subject.

10. Valet teaches a flow cytometry method that uses triple matrix classifiers, that is, groups of three antibodies specific for three different cellular antigens analyzed as a single parameter in three separate samples, to generate a triple matrix database. Thus, Valet fails to teach the claimed method that comprises concurrent analysis of the separate interactions between the immunoglobulins of the array and at least seven cell surface marker antigens. Valet lacks any suggestion that modification of the procedures disclosed therein is desirable; Valet teaches that a triple matrix pattern of antigen expression, even a single triple matrix pattern (*i.e.*, three antibodies specific for three different antigens), meets the criteria set forth for classification of several leukaemias and lymphomas (*see e.g.*. Valet, pages 286-287).

11. Terstappen fails to teach concurrent analysis of each immunoglobulin/antigen binding interaction and instead teaches a *sequential* analysis of *antibody pairs determined in sequential analyses by flow cytometry of at least 5 aliquots of a cell preparation taken from a patient*. Terstappen further teaches that the sequence of cell aliquot analysis and antibody pairs is important for practising the method disclosed therein (*see* Terstappen, column 2, lines 51-65). Thus, Terstappen teaches a method for classifying leukemias that uses different techniques and analyses and does not remotely suggest any desirability to combine the teachings therein with any other prior art to achieve the Applicants' invention.

12. Verwer fails to teach or suggest concurrent analysis of multiple individual antibody/antigen interactions and instead teaches multiple (8) sequential analyses for each blood or bone marrow sample of antibody/antigen pairs for fluoresoence and light scatter determined by flow cytometry (*see* Verwer throughout). Moreover, Verwer

teaches a technique for specifically analyzing flow cytometry data, wherein the technique provides positional information of cell clusters that is matched across multiple aliquots of a sample (Verwer, column 3, lines 7-11). This method uses one of several statistical clustering algorithms preferably modified mutual nearest neighbour values. Thus, Verwer also fails to provide any teaching, suggestion, or motivation to combine or modify the teachings of the prior art to produce the Applicants' claimed method. Verwer teaches a method for classifying leukemias that uses different techniques and analyses and does not suggest any desirability to combine the teachings therein with any other prior art to achieve the Applicants' method for identifying a type of leukemia in a human.

13. Orfao de Matos Correira E Vale teaches a flow cytometry method for distinguishing the major types of human lymphocytes, T, B, and NK cells and subsets of these cell types, by expression of certain CD antigens. A mixture of 5 monoclonal antibodies conjugated with 3 different fluorochromes is used in 6 combinations on 6 aliquots of cells to determine by flow cytometry 12 lymphoid sub-populations. Expression of CD antigens as taught in Orfao de Matos Correira E Vale, such as CD3, CD4, and CD8, on lymphocytes and detection of these antigens with antibodies has been long known in the immunology art. Orfao de Matos Correira E Vale fails to teach or suggest that the CD antigen/antibody interactions disclosed therein may be applicable to any method for identifying a type of leukemia in a human subject. Orfao de Matos Correira E Vale teaches a method for identifying subsets of lymphocytes using a different technique and analysis than the claimed method and does not suggest any desirability to combine the teachings therein with any other prior art to achieve the Applicants' method for identifying a type of leukemia in a human.

14. With respect to the rejection of the claims over Hoeffler in view of Terstappen, I submit that a person having ordinary skill in the art at the time of filing USSN '959 would not have been motivated by any suggestion or teaching in either document to combine the teachings therein to achieve the claimed method. Hoeffler describes a method for screening very large numbers of uncharacterized antibodies for those specific for a given antigen on a protein. Thus, the antibodies of the array are unknown, rather than the antigens in the applied protein sample. Hoeffler also described

a microarray of uncharacterized antibodies used to compare expression profiles of cells. Note that in both these applications, proteins or lysates rather than intact cells are used, and the identities of the antibodies in the array are unknown. There is no discussion of a pattern of recognition for leukemia, or a disease signature. Rather, mention was made on P30 L28 of "a population of antibodies diagnostic for a variety of disorders on a single surface." The only example supported by data involving a microarray of known antibodies and a cell extract was example VII (P46). In this example, 8 antibodies were used to make an array that was used to detect a single protein, β-galactosidase, in an extract of CHO cells (Fig. 5). Hoeffler is silent regarding contacting immunoglobulin molecules of an array with a biological sample containing cells for detection of the interaction between the immunoglobulin and the cell surface antigen. Hoeffler as a whole teaches a method for detecting an antibody or an antigen, wherein the antigen may be purified or partially purified (Hoeffler, paragraph 42).

15. Terstappen fails to teach concurrent analysis of each immunoglobulin/antigen binding interaction and instead teaches a *sequential* analysis of *antibody pairs*. The patent teaches that according to the method described therein, the sequence of the cell aliquot analysis and antibody pairing is important (*see* Terstappen, column 2, lines 51-65).

16. None of the documents alone or in combination teach the presently claimed method. Furthermore, none of the documents indicates any desirability to modify either method disclosed therein to achieve the Applicants' claimed method. Hoeffler fails to suggest, teach, or motivate a person having ordinary skill in the art to combine its teachings with any other prior art teaching to obtain the claimed method for identifying a type of leukemia. Terstappen also fails to provide any teaching, suggestion, or motivation to combine or modify the teachings of the prior art to produce the Applicants' claimed method. Terstappen teaches a method for classifying leukemias that uses different techniques and analyses and does not remotely suggest any desirability to combine the teachings therein with any other prior art to achieve the Applicants' invention.

17. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

R. I. Christopherson — 28 September 2004

RICHARD IAN CHRISTOPHERSON — Date

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